

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C12N 15/85, 15/49, 5/16, C12P 21/00,</b> <b>A61K 39/21</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 96/40953</b> <b>(43) International Publication Date:</b> 19 December 1996 (19.12.96)
<b>(21) International Application Number:</b> PCT/US96/08639 <b>(22) International Filing Date:</b> 3 June 1996 (03.06.96) <b>(30) Priority Data:</b> 08/479,703 7 June 1995 (07.06.95) US <b>(71) Applicant:</b> AMERICAN HOME PRODUCTS CORPORATION [US/US]; Five Giralda Farms, Madison, NJ 07940-0874 (US). <b>(72) Inventors:</b> CHAVEZ, Lloyd, George, Jr.; 8502 S. Forrest Street, Highlands Ranch, CO 80126 (US). WASMOEN, Terri; 1113 25th Avenue North, Fort Dodge, IA 50501 (US). HUANG, Chenglin; Apartment #3, 1454 North 31st Street, Fort Dodge, IA 50501 (US). <b>(74) Agents:</b> MANDEL, Adley, F.; American Home Products Corporation, Five Giralda Farms, Madison, NJ 07940-0874 (US) et al.		<b>(81) Designated States:</b> AL, AM, AU, BB, BG, BR, CA, CN, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, PT, RO, SG, SI, SK, TR, TT, UA, UZ, VN, ARIPO patent (KE, LS, MW, SD, SZ, UG), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> GENETICALLY ALTERED FELINE IMMUNODEFICIENCY VIRUSES AND THEIR USE AS AN EFFECTIVE VACCINE AGAINST FELINE IMMUNODEFICIENCY VIRUS INFECTION		
<b>(57) Abstract</b> <p>The present invention pertains to the prevention or lessening of disease in cats caused by Feline Immunodeficiency Virus (FIV). Prevention or lessening of disease is understood to mean the amelioration of any symptoms, including immune system disruptions, that result from FIV infection. The invention provides for a plasmid which encodes the FIV genome where said genome has had a portion of the gag gene, specifically the p10 (nucleocapsid) coding region, or a portion thereof, deleted. This deletion prevents the production of functional or whole p10 protein which, in turn, prevents the packaging of RNA into virions produced from transfection of this plasmid into an appropriate host cell, resulting in virions which do not contain RNA. Such virions will be described as "empty" virions. The invention also encompasses host cells transformed with the plasmid which produce the empty virions, and the empty virions themselves. In another embodiment, the invention encompasses vaccines that comprise one or more empty virions described above, with a pharmaceutically acceptable carrier or diluent and a pharmaceutically acceptable adjuvant. In yet another aspect, the invention provides methods for preventing or lessening disease caused by FIV, which is carried out by administering to a feline in need of such treatment the vaccines described above.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AM	Armenia	GB	United Kingdom	MW	Malawi
AT	Austria	GE	Georgia	MX	Mexico
AU	Australia	GN	Guinea	NE	Niger
BB	Barbados	GR	Greece	NL	Netherlands
BE	Belgium	HU	Hungary	NO	Norway
BF	Burkina Faso	IE	Ireland	NZ	New Zealand
BG	Bulgaria	IT	Italy	PL	Poland
BJ	Benin	JP	Japan	PT	Portugal
BR	Brazil	KE	Kenya	RO	Romania
BY	Belarus	KG	Kyrgyzstan	RU	Russian Federation
CA	Canada	KP	Democratic People's Republic of Korea	SD	Sudan
CF	Central African Republic	KR	Republic of Korea	SE	Sweden
CG	Congo	KZ	Kazakhstan	SG	Singapore
CH	Switzerland	LI	Liechtenstein	SI	Slovenia
CI	Côte d'Ivoire	LK	Sri Lanka	SK	Slovakia
CM	Cameroon	LR	Liberia	SN	Senegal
CN	China	LT	Lithuania	SZ	Swaziland
CS	Czechoslovakia	LU	Luxembourg	TD	Chad
CZ	Czech Republic	LV	Latvia	TG	Togo
DE	Germany	MC	Monaco	TJ	Tajikistan
DK	Denmark	MD	Republic of Moldova	TT	Trinidad and Tobago
EE	Estonia	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	UG	Uganda
FI	Finland	MN	Mongolia	US	United States of America
FR	France	MR	Mauritania	UZ	Uzbekistan
GA	Gabon			VN	Viet Nam

**GENETICALLY ALTERED FELINE IMMUNODEFICIENCY VIRUSES AND  
THEIR USE AS AN EFFECTIVE VACCINE AGAINST  
FELINE IMMUNODEFICIENCY VIRUS INFECTION**

**FIELD OF THE INVENTION**

The present invention pertains to the prophylaxis and treatment of disease caused by feline immunodeficiency virus (FIV), using genetically altered FIV virions. Specifically, a portion of the p10 gene, which encodes a protein responsible for packaging of the RNA into the virion, has been deleted. The resulting virions are produced in appropriate host cell lines and used to make vaccines comprising whole killed virions which do not comprise viral RNA.

**BACKGROUND OF THE INVENTION**

Feline immunodeficiency virus (FIV) infection is a significant health problem for domestic cats around the world. As in its human counterpart, infection with FIV causes a progressive disruption in immune function. In the acute phase of infection, the virus causes transient illness associated with symptoms such as lymphadenopathy, pyrexia, and neutropenia. Subsequently, an infected animal enters an asymptomatic phase of 1-2 years before clinical manifestations of immune deficiency become apparent, after which the mean survival time is usually less than one year.

FIV is a typical retrovirus that contains a single-stranded polyadenylated RNA genome, internal structural proteins derived from the *gag* gene product, and a lipid envelope containing membrane proteins derived from the *env* gene product (Bendinelli et al., *Clin. Microbiol. Rev.* 8:87, 1995). The *gag* gene is translated into a primary product of

about 50 kDa that is subsequently cleaved by a viral protease into the matrix (p15), capsid (p25), and nucleocapsid (p10) proteins. The start and the end for each cleavage product of the GAG polypeptide are indicated in Figure 2 underneath the open reading frame. The *env* gene yields a primary translation product of 75-80 kDa (unglycosylated molecular weight); in infected cells, the precursor has an apparent molecular weight of 145-150 kDa due to N-linked glycosylation. The *env* precursor is cleaved in the Golgi apparatus into the SU and TM proteins (also designated gp95 and gp40, respectively).

As discussed above, the *gag* gene of the feline immunodeficiency virus (FIV) is initially translated as a precursor polypeptide which is cleaved to yield the functionally mature matrix protein, capsid protein and nucleocapsid protein making up the core of virus (Elder et al., J. Virol. 67: 1869-76, 1993). The *pol* gene overlaps the *gag* gene by 112 nucleotides, and is in a -1 reading frame with respect to that of the *gag* gene.

Thus, the gene is translated as a Gag-Pol fusion protein produced by ribosome frameshifting. The overlapping region contains frameshift signals, GGGAAAC and GGAGAAAC, located at the 3' end of the *gag* gene (Morikawa et al., Virol. 186: 389-97, 1992).

The nucleocapsid protein, or p10, is a small basic protein, which is associated with the genomic RNA and may be required for viral RNA packaging (Egberink et al. J. Gen. Virol. 71: 739-743, 1990; Steinman et al., J. Gen. Virol. 71: 701-06, 1990). The p10 protein contains two cysteine arrays each consisting of 14 amino acid residues with the sequence C-X<sub>2</sub>-C-X<sub>4</sub>-H-X<sub>4</sub>-C (where X represents any amino acid and the subscript is the number of residues). Genetic studies with other retroviruses have shown that these two cysteine arrays are essential for viral RNA packaging (Rein et al., J. Virol. 68: 6124-29, 1994; Meric et al., J. Virol. 62: 3328-33; Gorelick et al., Proc. Natl. Acad. Sci. USA 85:8420-24, 1988). Therefore, deletion of these two cysteine arrays should, in theory, generate FIV virus particles which contains all viral proteins, but no viral genomic RNA. These FIV viral particles should be non-infectious and could be used to effect efficacious immune protection in vaccinated cats.

Most vaccines against FIV have failed to induce protective immunity. Ineffective vaccines have involved inactivated whole virus, fixed infected cells, recombinant CA and SU proteins, and a synthetic peptide corresponding to the V3 region

of SU. In some cases, the vaccine actually enhanced infection after challenge. In one system, vaccination with paraformaldehyde-fixed virus or infected cells resulted in protective immunity (Yamamoto et al., J. Virol. 67:601, 1993), but application of this approach by others was unsuccessful (Hosie et al., in Abstracts of the International Symposium on Feline Retrovirus Research, 1993, page 50).

Thus, there is a need in the art for an effective whole killed virion vaccine against FIV.

### **SUMMARY OF THE INVENTION**

The present invention pertains to the prevention or lessening of disease in cats caused by Feline Immunodeficiency Virus (FIV). Prevention or lessening of disease is understood to mean the amelioration of any symptoms, including immune system disruptions, that result from FIV infection.

The invention provides for a plasmid which encodes the FIV genome where said genome has had a portion of the *gag* gene, specifically the p10 (nucleocapsid) coding region, or a portion thereof, deleted. This deletion prevents the production of functional or whole p10 protein, which in turn, prevents the packaging of RNA into virions produced from transfection of this plasmid into an appropriate host cell, resulting in virions which do not contain RNA. Such virions will be described as "empty" virions. The invention also encompasses host cells transformed with the plasmid which produce the empty virions, and the empty virions themselves.

In another embodiment, the invention encompasses vaccines that comprise one or more empty virions described above, with a pharmaceutically acceptable carrier or diluent and a pharmaceutically acceptable adjuvant.

In yet another aspect, the invention provides methods for preventing or lessening disease caused by FIV, which is carried out by administering to a feline in need of such treatment the vaccines described above.

### **BRIEF DESCRIPTION OF THE DRAWINGS**

Figure 1 is a graphic illustration of the cloning strategy for creating FIV with deletion of p10.

Figure 2 shows the DNA sequence of the *gag* gene of FIV, [SEQ. I.D. NO. 5] with the delineations of the coding sequence for the various proteolytic products indicated. The double underlined DNA sequence is deleted in a preferred embodiment of the present invention.

Figure 3 shows the protein sequences for the translation products of the *gag* gene of FIV, including both the primary [SEQ. I.D. NO. 6] and secondary [SEQ. I.D. NO. 7] open reading frames. The double underlined amino acids are not encoded by a preferred embodiment of the present invention.

### **DETAILED DESCRIPTION OF THE INVENTION**

All patents, patent applications, and references cited herein are hereby incorporated by reference in their entirety. In the case of inconsistencies, the present disclosure, including definitions, will control.

The vaccine of the present invention may be prepared by creating a recombinant FIV carrying a deletion of the p10 gene, or a portion thereof, encoding a portion of the *gag* protein of Feline Immunodeficiency Virus (FIV). The cloning scheme employed to produce the deleted virus eliminates 39 codons which include the two cysteine arrays within the p10 gene without disrupting either the *gag* gene open reading frame or the *gag-pol* frameshifting as occurs in the wild type virus-infected cells. The two cysteine arrays are highlighted in Figure 2, where cysteine array 1 encompasses nucleotides 1129 to 1170 and cysteine array 2 encompasses nucleotides 1186 to 1227. The thirty nine codons and amino acids which are deleted are double underlined in Figures 2 and 3. The deletion does not disrupt the original p10 open reading frame. The deletion also does not alter the *gag-pol* frameshift start site and frameshift signal. Therefore, in theory, the frequency of *gag-pol* frameshifting at nucleotide 1242 should not be affected by the deletion of the 39 codons preceding the *gag-pol* frameshift start site. Figure 2 indicates the *gag-pol* frameshift start site by single underlining. Figure 2 indicates the 5' end of the POL polyprotein underneath the p10 open reading frame, while Figure 3 lists the amino acid sequence of p10 and the frameshifted POL protein.

The process for constructing the p10 deletion vaccine is outlined as follows. A plasmid construct is made which deletes a portion of the p10 encoding gene sequences

using PCR-mediated mutagenesis. The construct is designed to not delete any of the 112 nucleotides (1243 to 1353) which overlap the *gag* and *pol* genes and to not eliminate the frameshift signal which is necessary for *pol* transcription. Once constructed, the plasmid is transfected into an appropriate host cell, such as mammalian cells, and the transformed cells are screened for non-infectious virus production. Cells which prove to produce non-infectious (presumably empty) virions are used to produce high levels of virus particles, which are isolated from the cell culture medium.

Although this particular construct and method are effective in producing empty virions, i.e., those which do not contain RNA, one of ordinary skill in the art would recognize alternative well-known methods of achieving the same goal. For example, the deletion need not eliminate the whole p10 encoding sequence, only enough sequence for the function of the protein to be eliminated. One representative example of this approach would be deletion of only one of the two cysteine arrays. Further, fragments of sequence need not be deleted. Any genetic alteration, i.e., site-directed mutagenesis of cysteines within the array, using methods well known in the art can be employed to construct a FIV genome which encodes empty virions. Thus, well-known variants of the genetic alterations presently employed which result in genomes which encode empty virions are contemplated to be within the scope of the present invention.

The isolated virus may be stored after concentration at 4°C or frozen (-50°C or colder) or lyophilized until the time of use. Compounds such as NZ-amine, dextrose, gelatin or others designed to stabilize the virus during freezing and lyophilization may be added. The virus may be concentrated using commercially available equipment. To produce the vaccine, isolated particles can be chemically treated to ensure lack of infectivity, that is, inactivated and mixed with an adjuvant(s).

Typically, the concentration of virus in the vaccine formulation will be a minimum of  $10^{6.0}$  virus particles per dose, but will typically be in the range of  $10^{6.0}$  to  $10^{8.0}$  virus particles per dose. At the time of vaccination, the virus is thawed (if frozen) or reconstituted (if lyophilized) with a physiologically-acceptable carrier such as deionized water, saline, phosphate buffered saline, or the like. An additional optional component of the present vaccine is a pharmaceutically acceptable adjuvant. Non-limiting examples of suitable adjuvants include squalane and squalene (or other oils of animal origin); block

copolymers such as Pluronic® (L121) Saponin; detergents such as Tween -80; Quil® A, mineral oils such as Drakeol or Marcol®, vegetable oils such as peanut oil; Corynebacterium-derived adjuvants such as corynebacterium parvum; Propionibacterium-derived adjuvants such as Propionibacterium acne; Mycobacterium bovis (Bacillus Calmette and Guérin, or BCG); interleukins such as interleukin 2 and interleukin-12; monokines such as interleukin 1; tumor necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminum hydroxide or Quil®-A aluminum hydroxide; liposomes; iscom adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as muramyl dipeptides or other derivatives; Avridine; Lipid A; dextran sulfate; DEAE-Dextran or DEAE-Dextran with aluminum phosphate; carboxypolymethylene, such as Carbopol®; ethylene maleic anhydride (EMA); acrylic copolymer emulsions such as Neocryl® A640 (e.g. U.S. Patent 5,047,238); vaccinia or animal poxvirus proteins; subviral particle adjuvants such as orbivirus; cholera toxin; dimethyldioctadecylammonium bromide; or mixtures thereof.

Individual genetically altered virions may be mixed together for vaccination. Furthermore, the virus may be mixed with additional inactivated or attenuated viruses, bacteria, or fungi such as feline leukemia virus, feline panleukopenia virus, feline rhinotracheitis virus, feline calicivirus, feline infectious peritonitis virus, feline *Chlamydia psittaci*, *Microsporium canis*, or others. In addition, antigens from the above-cited organisms may be incorporated into combination vaccines. These antigens may be purified from natural sources or from recombinant expression systems, or may comprise individual subunits of the antigen or synthetic peptides derived therefrom.

The produced vaccine can be administered to cats by subcutaneous, intramuscular, oral, intradermal, or intranasal routes. The number of injections and their temporal spacing may be varied. One to three vaccinations administered at intervals of one to three weeks are usually effective.

The efficacy of the vaccines of the present invention is assessed by the following methods. At about one month after the final vaccination, vaccinates and controls are each challenged with 3 - 20 cat ID<sub>50</sub> units, preferably 5 cat ID<sub>50</sub> units of FIV, preferably the NCSU-1 isolate (ATCC accession number VR 2333). Whole blood is obtained from the



animals immediately before challenge, and at intervals after challenge, for measurement of a) viremia and b) relative amounts of CD4 and CD8 lymphocytes.

Viremia is measured by isolating mononuclear cells from the blood, and co-culturing the cells with mononuclear cells from uninfected animals. After 7 days of culture, the culture supernatants are tested for FIV by enzyme-linked immunoassay (See Example 3 below).

The ratio of CD4 to CD8 lymphocytes in the circulation of vaccinates and controls is taken as a measure of immune function. Typically, FIV infection causes an inversion of the normal CD4:CD8 ratio of about 1.5-4 to a pathological ratio of about 0.5-1. The titers of CD4 and CD8 lymphocytes are measured by flow cytometry using specific antibodies (see Example 3 below).

Another measure of immune function is to challenge vaccinates and controls with *Toxoplasma gondii* at 6 months - 12 months after the final vaccination. Normally, the severity of *T. gondii*-induced disease symptoms is considerably exacerbated in FIV-infected cats relative to uninfected cats. The severity of the *T. gondii* effect is determined by scoring ocular discharge, nasal discharge, dyspnea, and fever.

It will be understood that amelioration of any of the symptoms of FIV infection is a desirable clinical goal. This includes a lessening of the dosage of medication used to treat FIV-induced symptoms.

The following examples are intended to illustrate the present invention without limitation thereof.

#### **Example 1: Preparation of p10 deleted FIV strain**

##### **A. Isolation of Parental DNA**

Purified lambda DNA containing the full length proviral sequence for the NCSU-1 isolate is prepared with Wizard Lambda Preps DNA Purification System (Promega Corporation, Madison, WI) and is used as the parental DNA for constructing deletion mutants. DNA digestion, ligation and other molecular techniques are performed as described (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory, 1989).

### B. Preparation of FIV-Left Plasmid

Purified lambda DNA is digested with *Sall* to release the 11-kb insert DNA containing the full length FIV proviral sequence. The insert DNA is purified by the glass bead method using the GENECLAN II kit from BIO 101, Inc. and digested with *NcoI* which cuts only once on the FIV genome, producing a 2.9 kb *Sall*-*NcoI* fragment, designated as fragment A, and a 8.1 kb *NcoI*-*Sall* fragment, designated as fragment B.

Fragment A is purified by glass bead method as above and subcloned into plasmid vector pGEM 5Zf(t) (Promega Corp., Madison, WI) to generate plasmid pFIV-left. The plasmid pFIV-left contains the left portion of the viral genome including the LTR, p15, p25 and p10 gene.

### C. Deletion of p10 Sequence

Deletion of the two cysteine arrays within the p10 gene is facilitated by PCR-mediated mutagenesis using high-fidelity *Pwo* DNA polymerase according to the manufacturer's manual (Boehringer Mannheim, USA, Indianapolis, IN). The plasmid pFIV-left is used as the initial template for PCR reaction. SP6 primer and primer A are used to amplify 2.2-kb fragment C with sequence which ends at nucleotide 1124. The SP6 primer:

5'-TTAGGTGACACTATAGAATACTCAA-3' [SEQ. I.D. NO. 1]

anneals to the vector sequence upstream the *Sall* site. Primer A:

5'-GGTCCTGATCCTTTTGATTGCACTA-3' [SEQ. I.D. NO. 2]

anneals to the FIV sequence, nucleotides 1100 to 1124.

Primer B and T7 primer are used to amplify 0.6-kb fragment D which starts at nucleotide 1242. The primer:

5'-AAAGAATTCGGGAAACTGGAAGGCGG-3' [SEQ. I.D. NO. 3]

anneals within the gag p10 gene, nucleotides 1242 to 1267. The T7 primer:

5'-TAATACGACTCACTATAGGGCGAATTG-3' [SEQ. I.D. NO. 4]

anneals to the vector sequence downstream from the *NcoI* site.

The location for each GAG-specific primer is highlighted in Figure 2.

Fragment C and fragment D are purified as above, ligated and the ligation products are used as the template to amplify a 2.8-kb fragment using SP6 primer and T7 primer.

The 2.8-kb fragment generated is purified as above and digested with Sall and NcoI to generate fragment E. Fragment E is identical to fragment A except the sequence for the segment spanning the two cysteine arrays is deleted, i.e. the sequence spanning nucleotides 1125 to 1241 is removed (see Fig. 1).

#### **D. Construction of FIV delta p10 Plasmid**

Fragment E and fragment B generated are purified as above. Then fragment E and fragment B are combined and cloned into the Sall site of the gene targeting vector pMC1neo Poly A (Stratagene, LaJolla, CA; Thomas, K. R., and Capecchi, M. R., Cell 51: 503-21, 1987), generating plasmid pFIV delta p10. The plasmid pFIV delta p10 contains the entire FIV genome with internal deletion within the p10 gene in addition to the neomycin resistance gene present on the gene targeting vector.

#### **E. Production of Virions**

Stable transfectants are obtained by transfecting the plasmid pFIV delta p10 into Vero cells (ATCC CCL 81), Crandell feline kidney cells (ATCC CCL 94) or AH927 feline embryonic fibroblast cells (Overbaugh et al., Virol. 188: 558-569, 1992) and selection by G418 by using cationic liposome-mediated transfection with the LIPOFECTamine® reagent and G418 (Genticin) according to the manufacturer's instruction (Life Technologies, Inc., Gaithersburg, MD). Cultures of G418-resistant cells are tested for virus particle production by a) assaying the viral particle-associated reverse transcriptase activity; b) complementation plaque assay as described (Rein et al., J. Virol. 29: 494-500, 1979) to determine if the virus particles are able to initiate single cycle of infection; c) Western blotting using antiserum against the major core protein p25 (IDEXX, USA, Portland, ME) to examine the integrity of the viral proteins; and d) direct examination of viral particles by electron microscopy.

The virus particles released from the stably transfected cells are to be examined for a) absence of viral RNA and DNA by RT-PCR and DNA PCR and b) absence of infectivity by the standard validated infectivity assays.

#### **Example 2: Preparation of Whole Killed Empty FIV Vaccines**

Stably-transfected cells which produce non-infectious viral particles are grown on microcarriers in bioreactors or in roller bottles. Culture fluids are harvested at the time or multiple times when the viral particles reach high levels as determined by electron microscopy and/or the feline immunodeficiency virus antigen test kit (IDEXX, USA, Portland, ME). The viral particles are inactivated by treatment with formalin or with binary ethylenimine, according to standard protocols well known in the art. Following inactivation, the viral particles are concentrated 10 to 50 fold with the hollow fiber procedure using a cut-off at molecular weight of 10,000 to 100,000 daltons. For preparing the vaccines, the concentrated fluids containing viral particles are mixed with immunologically stimulating adjuvant, for example, ethylene maleic anhydride (EMA) 31, neocryl, MVP emulsigen, mineral oil, or adjuvant A or combination of several immunologically stimulating adjuvants. Adjuvant A is an adjuvant comprising a block copolymer, such as a polyoxypropylene-polyoxyethylene (POP-POE) block copolymer, preferably Pluronic® L121 (e.g. U.S. Patent 4,772,466), and an organic component, such as a metabolizable oil, e.g. an unsaturated turpin hydrocarbon, preferably squalane (2,6,10,15,19,23-hexamethyltetracosane) or squalene.

In this adjuvant mixture, the block copolymer, organic oil, and surfactant may be present in amounts ranging from about 10 to about 40 ml/L, about 20 to about 80 ml/L, and about 1.5 to about 6.5 ml/L, respectively. In a preferred embodiment of the stock adjuvant, the organic component is squalane present in an amount of about 40 mL/L, the surfactant is polyoxyethylenesorbitan monooleate (Tween®-80) present in an amount of about 3.2 ml/L, and the POP-POE block copolymer is Pluronic® L121 present in an amount of about 20 ml/L. Pluronic® L121 is a liquid copolymer at 15-40 C, where the polyoxypropylene (POP) component has a molecular weight of 3250 to 4000 and the polyoxyethylene (POE) component comprises about 10-20%, preferably 10%, of the total molecule.

Non-limiting examples of other suitable adjuvants include squalane and squalene (or other oils of animal origin); block copolymers such as Pluronic® (L121) Saponin; detergents such as Tween®-80; Quil® A, mineral oils such as Drakeol® or Marcol®, vegetable oils such as peanut oil; Corynebacterium-derived adjuvants such as corynebacterium parvum; Propionibacterium-derived adjuvants such as Propionibacterium

acne; *Mycobacterium bovis* (Bacillus Calmette and Guérin, or BCG); interleukins such as interleukin 2 and interleukin-12; monokines such as interleukin 1; tumor necrosis factor; interferons such as gamma interferon; combinations such as saponin-aluminum hydroxide or Quil®-A aluminum hydroxide; liposomes; iscom adjuvant; mycobacterial cell wall extract; synthetic glycopeptides such as muramyl dipeptides or other derivatives; Avridine; Lipid A; dextran sulfate; DEAE-Dextran or DEAE-Dextran with aluminum phosphate; carboxypolymethylene, such as Carbopol®; EMA; acrylic copolymer emulsions such as Neocryl® A640 (e.g. U.S. Patent 5,047,238); vaccinia or animal poxvirus proteins; subviral particle adjuvants such as orbivirus; cholera toxin; dimethyldioctadecylammonium bromide; or mixtures thereof. The composition may also include a non-ionic detergent or surfactant, preferably a polyoxyethylene sorbitan monooleate such as a Tween® detergent, most preferably Tween®-80, i.e. polyoxyethylene (20) sorbitan monooleate.

Typically, 1 ml dose contains at least  $10^6$  viral particles, as determined by electron microscopy or the feline immunodeficiency virus antigen test kit (IDEXX, USA, Portland, ME).

### **Example 3: Test of Efficacy of Whole Killed Empty FIV Vaccines**

#### **A. Vaccination**

Cats of age 8 weeks or greater are injected subcutaneously or intramuscularly with the vaccine prepared above. Each cat receives two injections of vaccine at a 2-4 week interval. Two to six weeks following vaccination, the vaccinated cats and non-vaccinated cats are challenged by inoculating with 5 cat ID<sub>50</sub> of feline immunodeficiency virus (NCSU-1 isolate (ATCC VR 2333) and some other isolates). Antibody response to vaccination is measured by ELISA using a neutralizing peptide within the immunodominant region (V3) of the FIV envelope protein (Lombardi et al., J. Virol. 67:4742-49, 1993). Viral replication following challenging is monitored biweekly by a) determining the levels of FIV RNA or/+ proviral DNA with RT-PCR and DNA PCR; and/or b) by co-cultivation for presence of infectious virus particles.

#### **1. Detection of Viremia**

##### **a. PCR Detection of FIV proviral DNA**

Mononuclear cells were isolated from whole blood using Percoll™ (Pharmacia Biotech, Piscataway NJ) gradients.  $5 \times 10^5$  cells were lysed and 1/10th of the lysate used in a polymerase chain reaction assay with oligonucleotide primers specific to the gag gene of FIV (TL Wasmoen et al. Vet. Immun. Immunopath. 35: 83-93, 1992) or the equivalent. FIV amplified DNA was detected by agarose gel electrophoresis and ethidium bromide staining or by enzyme linked oligonucleotide assays.

b. Tissue Culture Isolation of FIV

Culture isolate of FIV is performed as described previously (Wasmoen et al., Vet. Immun. Immunopath. 35:83-93, 1992). Mononuclear cells are isolated from whole blood using Percoll™ (Pharmacia Biotech, Piscataway NJ) gradients.  $5 \times 10^5$  cells from FIV-challenged cats were cultured with  $1 \times 10^6$  mononuclear cells isolated from uninfected cats. Cultures are fed with RPMI media every 7 days and supernatant tested for the presence of FIV by an enzyme-linked immunosorbent assay (ELISA) that detects FIV p25 antigen (Petcheck ELISA, IDEXX, Portland, ME). Alternatively, plasma can be used as the source of infectious virus.

2. Lymphocyte Subsets

Leukocytes are isolated from whole blood using Histopaque™ (Sigma Chemical Company, St. Louis MO) and lymphocyte subsets quantitated by staining the cells with antibodies specific to CD4 (monoclonal antibody CAT30A), CD8 (monoclonal antibody FLSM 3.357), pan T lymphocytes (monoclonal antibody FLSM 1.572) or B lymphocytes (anti-cat IgG) followed by FACS analysis. These monoclonal antibodies are described elsewhere (M.B. Tompkins et al. Vet. Immunol. Immunopathol. 26:305-317, 1990) and the flow cytometry procedure is the same as previously described (R.V. English et al. J. Infect. Dis. 170:543-552, 1994). CD4:CD8 ratios are calculated.

B. Toxoplasma gondii Challenge

Eight to twelve weeks following challenge with FIV, the cats are inoculated with 10,000 to 50,000 tachozoites of *Toxoplasma gondii*. Tachozoites of the ME49 strain of *T. gondii* that were frozen in 10% glycerol or oocysts were inoculated intraperitoneally into Swiss mice (Charles Rivers Laboratories) and serially passed in mice according to published

procedures (Davidson et al., *Am. J. Pathol.* **143**:1486, 1993). Tachozoites harvested from peritoneal fluids of mice were enumerated using a hemacytometer. Cats were tranquilized using ketamine hydrochloride and inoculated with 50,000 fresh tachozoites into the right common carotid artery that had been surgically isolated. Inoculation with *Toxoplasma* in this dosage generally causes mortality in up to 50% of cats which are FIV-infected and have not been vaccinated. Following *Toxoplasma* challenge, cats are monitored weekly for signs of clinical disease including ocular discharge, nasal discharge, dyspnea, fever, depression, and weight loss for 3 days prior to and up to 48 days following *T. gondii* inoculation.

Clinical signs follow *T. gondii* challenge were scored as follows:

Clinical Sign	Score
Fever	103.0 to 103.9°F 1 point per day
	104.0 to 104.9°F 2 points per day
	≥105.0°F 3 points per day
(Temperatures were not scored until ≥1°F above baseline.)	
Depression/Lethargy	1 point per day
Dehydration	2 points per day
Nasal Discharge	1 point per day
Ocular Discharge	1 point per day
Respiratory Distress:	
Tachypnea	2 points per day
Dyspnea	4 points per day

It is expected that the vaccine prepared as described above will significantly reduce the appearance of clinical signs and mortality due to *Toxoplasma* infection.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

- (i) APPLICANT: Wasmoen, Terri  
Chu, Hsien-Jue  
Chavez, Lloyd
- (ii) TITLE OF INVENTION: Recombinant Raccoon Pox Viruses and  
Their Use as an Effective Vaccine Against Feline  
Immunodeficiency Virus Infection
- (iii) NUMBER OF SEQUENCES: 14
- (iv) CORRESPONDENCE ADDRESS:
  - (A) ADDRESSEE: American Home Products Corporation
  - (B) STREET: 5 Giralda Farms
  - (C) CITY: Madison
  - (D) STATE: New Jersey
  - (E) COUNTRY: U.S.A.
  - (F) ZIP: 07940-0894
- (v) COMPUTER READABLE FORM:
  - (A) MEDIUM TYPE: Floppy disk
  - (B) COMPUTER: IBM PC compatible
  - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
  - (D) SOFTWARE: PatentIn Release #1.0, Version #1.30
- (vi) CURRENT APPLICATION DATA:
  - (A) APPLICATION NUMBER:
  - (B) FILING DATE:
  - (C) CLASSIFICATION:
- (viii) ATTORNEY/AGENT INFORMATION:
  - (A) NAME: Matthews, Gale F.
  - (B) REGISTRATION NUMBER: 32,269
  - (C) REFERENCE/DOCKET NUMBER: AHP-95065 PCT
- (ix) TELECOMMUNICATION INFORMATION:
  - (A) TELEPHONE: 201-660-6329
  - (B) TELEFAX: 201-660-7160
  - (C) TELEX: 125751

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Feline immunodeficiency virus
  - (B) STRAIN: 14
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: 6637-6659
  - (C) UNITS: bp
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

TATAGAAGCA CCCCAAGAAG AG



15

## (2) INFORMATION FOR SEQ ID NO:2:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iv) ANTI-SENSE: YES
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Feline immunodeficiency virus
  - (B) STRAIN: 14

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CATTCCCCCA AAGTTATATT TC

22

## (2) INFORMATION FOR SEQ ID NO:3:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 22 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Feline immunodeficiency virus
  - (B) STRAIN: 14
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: 8264-8285
  - (C) UNITS: bp

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTAGTTACAT TAGAGCATCA AG

22

## (2) INFORMATION FOR SEQ ID NO:4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 27 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (iv) ANTI-SENSE: YES
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Feline immunodeficiency virus
  - (B) STRAIN: PPR
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: 9126-9145
  - (C) UNITS: bp

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

TTCTAGATCT TCAGGGTCCC AATACTC

27

## (2) INFORMATION FOR SEQ ID NO:5:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Feline immunodeficiency virus
  - (B) STRAIN: 14
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: 610-630
  - (C) UNITS: bp

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CAATTCTAGA GAGACTCTAC AGCAACATG

29

## (2) INFORMATION FOR SEQ ID NO:6:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 29 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Feline immunodeficiency virus
  - (B) STRAIN: 14
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: 2005-2026
  - (C) UNITS: bp

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TAATAGATCT GGCCTCTTTT CTAATGATG

29

## (2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 23 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vi) ORIGINAL SOURCE:
  - (A) ORGANISM: Feline immunodeficiency virus
  - (C) INDIVIDUAL ISOLATE: NCSU-1
- (viii) POSITION IN GENOME:
  - (B) MAP POSITION: 471-493
  - (C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

TATGGAAAAG GCAAGAGAAG GAC

23

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Feline immunodeficiency virus
- (C) INDIVIDUAL ISOLATE: NCSU-1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: 763-785
- (C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

TCGAGATACC ATGCTCTACA CTG

23

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 24 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Feline immunodeficiency virus
- (C) INDIVIDUAL ISOLATE: NCSU-1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: 857-880
- (C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

TATGGAAAAG ATGGGATGAG ACTA

24

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 23 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: Feline immunodeficiency virus
- (C) INDIVIDUAL ISOLATE: NCSU-1

(viii) POSITION IN GENOME:

- (B) MAP POSITION: 1513-1535
- (C) UNITS: bp

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GTCACCTTACC TTCATAGTAA ACC

23

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 449 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(vi) ORIGINAL SOURCE:

(A) ORGANISM: Feline immunodeficiency virus

(C) INDIVIDUAL ISOLATE: NCSU-1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Met	Gly	Asn	Gly	Gln	Gly	Arg	Asp	Trp	Lys	Met	Ala	Ile	Lys	Arg	Cys	
1				5					10					15		
Ser	Asn	Ala	Ala	Val	Gly	Val	Gly	Gly	Lys	Ser	Lys	Lys	Phe	Gly	Glu	
			20					25					30			
Gly	Asn	Phe	Arg	Trp	Ala	Ile	Arg	Met	Ala	Asn	Val	Ser	Thr	Gly	Arg	
		35					40					45				
Glu	Pro	Gly	Asp	Ile	Pro	Glu	Thr	Leu	Asp	Gln	Leu	Arg	Leu	Val	Ile	
	50					55				60						
Cys	Asp	Leu	Gln	Glu	Arg	Lys	Lys	Phe	Gly	Ser	Cys	Lys	Glu	Ile		
65				70				75					80			
Asp	Lys	Ala	Ile	Val	Thr	Leu	Lys	Val	Phe	Ala	Ala	Val	Gly	Leu	Leu	
			85					90					95			
Asn	Met	Thr	Val	Ser	Ser	Ala	Ala	Ala	Ala	Glu	Asn	Met	Phe	Thr	Gln	
			100					105					110			
Met	Gly	Leu	Asp	Thr	Arg	Pro	Ser	Met	Lys	Glu	Ala	Gly	Gly	Lys	Glu	
	115					120						125				
Glu	Gly	Pro	Pro	Gln	Ala	Phe	Pro	Ile	Gln	Thr	Val	Asn	Gly	Val	Pro	
	130					135						140				
Gln	Tyr	Val	Ala	Leu	Asp	Pro	Lys	Met	Val	Ser	Ile	Phe	Met	Glu	Lys	
145					150					155					160	
Ala	Arg	Glu	Gly	Leu	Gly	Gly	Glu	Glu	Val	Gln	Leu	Trp	Phe	Thr	Ala	
			165					170						175		
Phe	Ser	Ala	Asn	Leu	Thr	Pro	Thr	Asp	Met	Ala	Thr	Leu	Ile	Met	Ala	
			180					185						190		
Ala	Pro	Gly	Cys	Ala	Ala	Asp	Lys	Glu	Ile	Leu	Asp	Glu	Ser	Leu	Lys	
		195					200					205				
Gln	Leu	Thr	Ala	Gly	Tyr	Asp	Arg	Thr	His	Pro	Pro	Asp	Ala	Pro	Arg	
	210					215					220					
Pro	Leu	Pro	Tyr	Phe	Thr	Ala	Ala	Glu	Ile	Met	Gly	Ile	Gly	Phe	Thr	
225					230					235					240	

## 19

Gln Glu Gln Gln Ala Glu Ala Arg Phe Ala Pro Ala Arg Met Gln Cys  
 245 250 255  
 Arg Ala Trp Tyr Leu Glu Gly Leu Gly Lys Leu Gly Ala Ile Lys Ala  
 260 265 270  
 Lys Ser Pro Arg Ala Val Gln Leu Arg Gln Gly Ala Lys Glu Asp Tyr  
 275 280 285  
 Ser Ser Phe Ile Asp Arg Leu Phe Ala Gln Ile Asp Gln Glu Gln Asn  
 290 295 300  
 Thr Ala Glu Val Lys Leu Tyr Leu Lys Gln Ser Leu Ser Met Ala Asn  
 305 310 315 320  
 Ala Asn Ala Glu Cys Lys Lys Pro Met Thr His Leu Lys Pro Glu Ser  
 325 330 335  
 Thr Leu Glu Glu Lys Leu Arg Ala Cys Gln Glu Ile Gly Ser Pro Gly  
 340 345 350  
 Tyr Lys Met Gln Leu Leu Ala Glu Ala Leu Thr Lys Val Gln Val Val  
 355 360 365  
 Gln Ser Lys Gly Ser Gly Pro Val Cys Phe Asn Cys Lys Lys Pro Gly  
 370 375 380  
 His Leu Ala Arg Gln Cys Arg Glu Val Arg Lys Cys Asn Lys Cys Gly  
 385 390 395 400  
 Lys Pro Gly His Val Ala Ala Lys Cys Trp Gln Gly Asn Arg Lys Asn  
 405 410 415  
 Ser Gly Asn Trp Lys Ala Gly Arg Ala Ala Ala Pro Val Asn Gln Val  
 420 425 430  
 Gln Gln Ala Val Met Pro Ser Pro Pro Met Glu Glu Lys Leu Leu Asp  
 435 440 445  
 Leu

## (2) INFORMATION FOR SEQ ID NO:12:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 855 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Feline immunodeficiency virus
- (C) INDIVIDUAL ISOLATE: NCSU-1

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Ala Glu Gly Phe Ala Ala Asn Arg Gln Trp Ile Gly Glu Glu Ala  
 1 5 10 15  
 Glu Glu Leu Leu Asp Phe Asp Ile Ala Thr Gln Met Asn Glu Glu Gly  
 20 25 30  
 Pro Leu Asn Pro Gly Met Asn Pro Phe Arg Val Pro Gly Ile Thr Asp  
 35 40 45

## 20

Lys Glu Lys Gln Asp Tyr Cys Asn Ile Leu Gln Pro Lys Leu Gln Asp  
 50 55 60  
 Leu Arg Asn Glu Leu Gln Glu Val Lys Leu Glu Gly Asn Ala Gly  
 65 70 75 80  
 Lys Phe Arg Arg Thr Arg Phe Leu Arg Tyr Ser Asp Glu Gln Val Leu  
 85 90 95  
 Ser Pro Val His Ala Phe Ile Gly Tyr Cys Ile Tyr Leu Gly Asn Arg  
 100 105 110  
 Asn Lys Leu Gly Ser Leu Arg His Asp Ile Asp Ile Glu Ala Pro Pro  
 115 120 125  
 Glu Glu Cys Tyr Asp Asn Arg Glu Lys Gly Thr Thr Asp Asn Ile Lys  
 130 135 140  
 Tyr Gly Arg Arg Cys Cys Leu Gly Thr Val Thr Leu Tyr Leu Ile Leu  
 145 150 155 160  
 Phe Ile Gly Leu Ile Ile Tyr Ser Gln Thr Ala Asp Ala Gln Val Val  
 165 170 175  
 Trp Arg Leu Pro Pro Leu Val Val Pro Val Glu Glu Ser Glu Ile Ile  
 180 185 190  
 Phe Trp Asp Cys Trp Ala Pro Glu Glu Pro Ala Cys Gln Asp Phe Leu  
 195 200 205  
 Gly Ala Met Ile His Leu Lys Ala Lys Thr Asn Ile Ser Ile Arg Glu  
 210 215 220  
 Gly Pro Thr Leu Gly Asn Trp Ala Arg Glu Ile Trp Ala Thr Leu Phe  
 225 230 235 240  
 Lys Lys Ala Thr Arg Gln Cys Arg Arg Gly Arg Ile Trp Lys Arg Trp  
 245 250 255  
 Asp Glu Thr Ile Thr Gly Pro Ser Gly Cys Ala Asn Asn Thr Cys Tyr  
 260 265 270  
 Asn Val Ser Ala Ile Val Pro Asp Tyr Gln Arg Tyr Leu Asp Arg Val  
 275 280 285  
 Asp Thr Trp Leu Gln Gly Lys Ile Asn Ile Ser Leu Cys Leu Thr Gly  
 290 295 300  
 Gly Lys Met Leu Tyr Asn Lys Val Thr Lys Gln Leu Ser Tyr Cys Thr  
 305 310 315 320  
 Asp Pro Leu Gln Ile Pro Leu Ile Asn Tyr Thr Phe Gly Pro Asn Gln  
 325 330 335  
 Thr Cys Met Trp Asn Thr Ser Gln Ile Gln Asp Pro Glu Ile Pro Gln  
 340 345 350  
 Cys Gly Trp Trp Asn His Met Ala Tyr Tyr Asn Ser Cys Lys Trp Glu  
 355 360 365  
 Glu Ala Lys Val Lys Phe His Cys Gln Arg Thr Gln Ser Gln Pro Gly  
 370 375 380  
 Ser Trp Arg Arg Ala Ile Ser Ser Trp Lys Gln Arg Asn Arg Trp Glu  
 385 390 395 400  
 Trp Arg Pro Asp Phe Glu Ser Glu Lys Val Lys Ile Ser Leu Gln Cys  
 405 410 415

21

Asn Ser Thr Lys Asn Leu Thr Phe Ala Met Arg Ser Ser Gly Asp Tyr  
 420 425 430  
 Gly Glu Val Thr Gly Ala Trp Ile Glu Phe Gly Cys His Arg Asn Lys  
 435 440 445  
 Ser Asn Leu His Thr Glu Ala Arg Phe Arg Ile Arg Cys Arg Trp Asn  
 450 455 460  
 Val Gly Ser Asp Thr Ser Leu Ile Asp Thr Cys Gly Asn Thr Pro Asn  
 465 470 475 480  
 Val Ser Gly Ala Asn Pro Val Asp Cys Thr Met Tyr Ser Asn Lys Met  
 485 490 495  
 Tyr Lys Phe Ser Leu Pro Asn Gly Phe Thr Met Lys Val Asp Asp Leu  
 500 505 510  
 Ile Met His Phe Asn Met Pro Lys Ala Val Glu Met Asn Asn Ile Ala  
 515 520 525  
 Gly Asn Trp Ser Cys Thr Ser Asp Leu Pro Ser Ser Trp Gly Tyr Met  
 530 535 540  
 Asn Cys Asn Cys Pro Asn Ser Ser Ser Ser Tyr Ser Gly Thr Lys Met  
 545 550 555 560  
 Ala Cys Pro Ser Asn Arg Gly Ile Leu Arg Asn Trp Tyr Asn Pro Val  
 565 570 575  
 Ala Gly Leu Arg Gln Ser Leu Glu Gln Tyr Gln Val Val Lys Gln Pro  
 580 585 590  
 Asp Tyr Leu Leu Val Pro Glu Glu Val Met Glu Tyr Lys Pro Arg Arg  
 595 600 605  
 Lys Arg Ala Ala Ile His Val Met Leu Ala Leu Ala Thr Val Leu Ser  
 610 615 620  
 Ile Ala Gly Ala Gly Thr Gly Ala Thr Ala Ile Gly Met Val Thr Gln  
 625 630 635 640  
 Tyr His Gln Val Leu Ala Thr His Gln Glu Ser Met Glu Lys Val Thr  
 645 650 655  
 Glu Ala Leu Glu Ile Asn Asn Leu Arg Leu Val Thr Leu Glu His Gln  
 660 665 670  
 Val Leu Val Ile Gly Leu Lys Val Glu Ala Met Glu Lys Phe Leu Tyr  
 675 680 685  
 Thr Ala Phe Ala Met Gln Glu Leu Gly Cys Asn Pro Asn Gln Phe Phe  
 690 695 700  
 Ser Lys Ile Pro Leu Glu Leu Trp Thr Arg Tyr Asn Met Thr Ile Asn  
 705 710 715 720  
 Gln Thr Ile Trp Asn His Gly Asn Ile Thr Leu Gly Glu Trp Tyr Asn  
 725 730 735  
 His Thr Lys Asp Leu Gln Pro Lys Phe Tyr Glu Ile Ile Met Asp Ile  
 740 745 750  
 Glu Pro Asn Asn Val Gln Gly Lys Thr Gly Ile Gln Gln Leu Pro Lys  
 755 760 765  
 Trp Glu Asp Trp Val Arg Trp Ile Gly Asn Ile Pro Gln Tyr Leu Lys  
 770 775 780

## 22

Gly Leu Leu Gly Gly Ile Leu Gly Ile Gly Leu Gly Val Leu Leu Leu  
 785 790 795 800  
 Ile Leu Cys Leu Pro Thr Leu Val Asp Cys Ile Arg Asn Cys Ile His  
 805 810 815  
 Lys Ile Leu Gly Tyr Thr Val Ile Ala Met Pro Glu Val Glu Gly Glu  
 820 825 830  
 Glu Ile Gln Pro Gln Met Glu Leu Arg Arg Asn Gly Ser Gln Phe Gly  
 835 840 845  
 Met Ser Glu Lys Glu Glu Glu  
 850 855

## (2) INFORMATION FOR SEQ ID NO:13:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1353 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Feline immunodeficiency virus
- (C) INDIVIDUAL ISOLATE: NCSU-1

## (viii) POSITION IN GENOME:

- (B) MAP POSITION: 1-1353
- (C) UNITS: bp

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

ATGGGGAATG GACAGGGGCG AGATTGGAAA ATGGCCATTA AGAGATGTAG TAATGCTGCT	60
GTAGGAGTAG GGGGGAAGAG TAAAAAATTT GGGGAAGGGA ATTCAGATG GGCCATTAGA	120
ATGGCTAATG TATCTACAGG ACGAGAACCT GGTGATATAC CAGAGACTTT AGATCAACTA	180
AGGTGCTTGA TTTGCGATTT ACAAGAAAGA AGAAAAAAT TTGGATCTTG CAAAGAAATT	240
GATAAGGCAA TTGTTACATT AAAAGTCTTT GCGGCAGTAG GACTTTTAAA TATGACAGTG	300
TCTTCTGCTG CTGCAGCTGA AAATATGTTC ACTCAGATGG GATTAGACAC TAGACCATCT	360
ATGAAAGAAG CAGGAGGAAA AGAGGAAGGC CCTCCACAGG CATTCCTAT TCAAACAGTA	420
AATGGAGTAC CACAATATGT AGCACTTGAC CAAAAAATGG TGTCCATTTT TATGGAAAAG	480
GCAAGAGAAG GATTAGGAGG TGAGGAAGTT CAGCTATGGT TCACTGCCTT CTCTGCAAAT	540
TTAACACCTA CTGACATGGC CACATTAATA ATGGCCGCAC CAGGGTGCGC TGCAGATAAA	600
GAAATATTGG ATGAAAGCTT AAAGCAACTT ACTGCAGGAT ATGATCGTAC ACATCCCCCT	660
GATGCTCCCA GACCATTACC CTATTTTACT GCAGCAGAAA TTATGGGTAT TGGATTTACT	720
CAAGAACAAC AAGCAGAAGC AAGATTTGCA CCAGCTAGGA TGCAGTGTAG AGCATGGTAT	780
CTCGAGGGAC TAGGAAAATT GGGCGCCATA AAAGCTAAGT CTCCTCGAGC TGTGCAGTTA	840
AGACAAGGAG CTAAGGAAGA TTATTCATCC TTTATTGACA GATTGTTTGC CCAAATAGAT	900
CAAGAACAAA ATACAGCTGA AGTTAAGTTA TATTTAAAC AGTCATTAAG CATGGCTAAT	960



23

GCTAATGCAG AATGTAAAAA GCCAATGACC CACCTTAAGC CAGAAAGTAC CCTAGAAGAA 1020  
 AAGTTGAGAG CTTGTCAAGA AATAGGCTCA CCAGGATATA AAATGCAACT CTTGGCAGAA 1080  
 GCTCTTACAA AAGTTCAAGT AGTGCAATCA AAAGGATCAG GACCAGTGTG TTTTAATTGT 1140  
 AAAAAACCAG GACATCTAGC AAGACAATGT AGAGAAGTGA GAAAATGTAA TAAATGTGGA 1200  
 AAACCTGGTC ATGTAGCTGC CAAATGTTGG CAAGGAAATA GAAAGAATTC GGGAAACTGG 1260  
 AAGGCGGGGC GAGCTGCAGC CCCAGTGAAT CAAGTGCAGC AAGCAGTAAT GCCATCTGCA 1320  
 CCTCCAATGG AGGAGAAACT ATTGGATTTA TAA 1353

## (2) INFORMATION FOR SEQ ID NO:14:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3225 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: DNA (genomic)

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Feline immunodeficiency virus
- (C) INDIVIDUAL ISOLATE: NCSU-1

## (viii) POSITION IN GENOME:

- (B) MAP POSITION: 1-3225
- (C) UNITS: bp

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GGATCCAACA ATAATTATGG CAGAAGGATT TGCAGCCAAT AGACAATGGA TAGGACCAGA 60  
 AGAAGCTGAA GAGTTATTAG ATTTTGATAT AGCAACACAA ATGAATGAAG AAGGGCCACT 120  
 AAATCCAGGG ATGAACCCAT TTAGGGTACC TGGGAATAACA GATAAAGAAA AGCAAGACTA 180  
 TTGTAACATA TTACAACCTA AGTTACAAGA TTTACGGAAT GAACTTCAAG AGGTAAACT 240  
 AGAAGAAGGA AATGCAGGTA AGTTTAGAAG AACAAGATTT TTAAGGTATT CTGATGAACA 300  
 AGTATTGTCC CCGGTTTCATG CGTTCATAGG ATATTGTATT TATTTAGGTA ATCGAAATAA 360  
 GTTAGGATCT TTAAGACATG ACATTGATAT TGAAGCACCC CCCGAAGAGT GTTATGATAA 420  
 TAGAGAGAAG GGTACAACTG ACAATATAAA ATATGGTAGA CGATGTTGCC TAGGAACGGT 480  
 GACTTTGTAC CTGATTTTAT TTATAGGATT AATAATATAT TCACAGACAG CCGACGCTCA 540  
 GGATAGTATGG AGACTTCCAC CATTAGTAGT CCCAGTAGAA GAATCAGAAA TAATTTTTTG 600  
 GGATTGTTGG GCACCAGAAG AACCCGCCTG TCAGGACTTT CTTGGGGCAA TGATACATCT 660  
 AAAAGCTAAG ACAAATATAA GTATACGAGA GGGACCTACC TTGGGGAATT GGGCTAGAGA 720  
 AATATGGGCA ACATTATTCA AAAAGGCTAC TAGACAATGT AGAAGAGGCA GAATATGGAA 780  
 AAGATGGGAT GAGACTATAA CAGGACCATC AGGATGTGCT AATAACACAT GTTATAATGT 840  
 TTCAGCAATA GTACCTGATT ATCAGCGTTA TTTAGATAGA GTAGATACTT GGTACAAGG 900  
 GAAAATAAAT ATATCATTAT GTCTAACAGG AGGAAAAATG TTGTACAATA AAGTTACAAA 960  
 ACAATTAAGC TATTGTACAG ACCCATTACA AATCCCACTG ATCAATTATA CATTGGACC 1020

TAATCAAACA TGTATGTGGA ATACTTCACA AATTCAGGAC CCTGAAATAC CACAATGTGG	1080
ATGGTGGAAT CACATGGCCT ATTATAACAG TTGTAAATGG GAAGAGGCAA AGGTAAAGTT	1140
TCATTGTCAA AGAACACAGA GTCAGCCTGG GTCATGGCGT AGAGCAATCT CGTCATGGAA	1200
ACAAAGAAAT AGATGGGAGT GGAGACCAGA TTTTGAGAGT GAAAAGGTGA AAATATCTCT	1260
ACAGTGCAAT AGCACGAAAA ACCTAACCTT TGCAATGAGA AGTTCAGGAG ATTATGGAGA	1320
AGTAACGGGA GCTTGGATAG AGTTTGGATG TCATAGAAAT AAATCAAACC TTCATACTGA	1380
AGCAAGGTTT AGAATTAGAT GTAGATGGAA TGTAGGGAGT GATACCTCGC TCATTGATAC	1440
ATGTGGAAAC ACTCCAAATG TTTCAGGTGC GAATCCTGTA GATTGTACCA TGTATTCAA	1500
TAAAATGTAC AAGTTTTCTT TACCAAACGG GTTTACAATG AAGGTAGATG ACCTTATTAT	1560
GCATTTCAAT ATGCCAAAAG CTGTAGAAAT GAATAATATT GCTGGAAATT GGTCTTGTAC	1620
ATCTGACTTG CCATCGTCAT GGGGGTATAT GAATTGTAAT TGCCCAAATA GTAGTAGTAG	1680
TTATAGTGGT ACTAAAATGG CATGTCCTAG CAATCGAGGC ATCTTAAGGA ATTGGTATAA	1740
CCCAGTAGCA GGATTACGAC AATCCTTAGA ACAGTATCAA GTTGTAAAAC AACCAGATTA	1800
CTTACTGGTC CCAGAGGAAG TCATGGAATA TAAACCTAGA AGGAAAAGGG CAGCTATTCA	1860
TGTTATGTTG GCTCTTGCAA CAGTATTATC TATTGCCGGT GCAGGGACGG GGGCTACTGC	1920
TATAGGGATG GTAACACAAT ACCACCAAGT TCTGGCAACC CATCAAGAAT CTATGGAAAA	1980
GGTGACTGAA GCCTTAGAGA TAAACAACCT AAGGTTAGTT ACATTAGAGC ATCAAGTACT	2040
AGTAATAGGA TTAAGTAG AAGCTATGGA AAAATTTTTA TATACAGCTT TCGCTATGCA	2100
AGAATTAGGA TGTAATCCAA ATCAATTTTT CTCCAAAATC CCTCTTGAGT TGTGGACAAG	2160
GTATAATATG ACTATAAATC AAACAATATG GAATCATGGA AATATAACTT TGGGGGAATG	2220
GTATAACCAC ACCAAAGATT TACAACCAA GTTTTATGAA ATAATAATGG ACATAGAACC	2280
AAATAATGTA CAAGGGAAAA CAGGGATACA ACAATTACCC AAGTGGGAAG ATTGGGTAAG	2340
ATGGATAGGA AATATTCCAC AATATTTAAA GGGACTATTG GGAGGTATCT TGGGAATAGG	2400
ATTAGGAGTG TTATTATTGA TTTTATGTTT ACCTACATTG GTTGATTGTA TAAGAAATTG	2460
TATCCACAAG ATACTAGGAT ACACAGTAAT TGCAATGCCT GAAGTAGAAG GAGAAGAAAT	2520
ACAACCACAA ATGGAATTGA GGAGAAATGG TAGCCAATTT GGCATGTCTG AAAAAGAGGA	2580
GGAATGATGA AGTATCTCAG ACTTATTTTA TAAGGGAGAT ACTGTGCTAA GTTCTTCCCT	2640
TTGAGGAAGG TATGTCATAT GAATCCATTT CGAACCAAAT CAACTAATA AAGTATGTAT	2700
TGTAAGGTAA AAGGAAAAGA CAAAGAAGAA GAAGAAAGAA GAAAGCTTTC AAGAGGATGA	2760
TGACAGAGTT AGAAGATCGC TTCAGGAAGC TATTTGGCAC GACTTCTACA ACGGGAGACA	2820
GCACAGTAGA TTCTGAAGAT GAACCTCCTA AAAAAGAAAA AAGGGTGGAC TGGGATGAGT	2880
ATTGGAACCC TGAAGAAATA GAAAGAATGC TTATGGACTA GGGACTGTTT ACGAACAAAT	2940
GATAAAAGGA AATAGCTAAG CATGACTCAT AGTTAAAGCG CTAGCAGCTG CTTAACCGCA	3000
AAACCACATC CTATGTAAAG CTTGCTAATG ACGTATAAGT TGTTCATTG TAAGAGTATA	3060
TAACCAAGTG TTGTGAAAC TTCGAGGAGT CTCTCCGTTG AGGACTTTTG AGTTCTCCCT	3120

WO 96/40953

PCT/US96/08639

25

TGAGGCTCCC ACAGATACAA TAAATATTTG AGATTGAACC CTGTCAAGTA TCTGTGTAAT 3180  
CTTTTTTACC TGTGAGGTCT CGGAATCCGG GCCGAGAACT TCGCA 3225

What is claimed is:

1. A plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof.
2. The plasmid of claim 1 wherein said deletion of nucleotides encoding the FIV p10 protein are as set out in Figure 2.
3. The plasmid of claim 2 wherein said deletion encompasses nucleotides which results in the deletion of amino acids 14 - 52 of the FIV p10 protein upon translation.
4. A vaccine comprising virions of Feline Immunodeficiency Virus (FIV) which do not comprise whole p10 nucleocapsid protein.
5. The vaccine of claim 4 further comprising a pharmaceutically acceptable adjuvant.
6. The vaccine of claim 4 wherein said virions are produced from transfection of appropriate host cells by a plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof and a pharmaceutically acceptable carrier or diluent.
7. The vaccine of claim 4 wherein said deletion of nucleotides encoding the FIV p10 protein is as set out in Figure 2.
8. The vaccine of claim 4 wherein said deletion encompasses nucleotides which results in the deletion of amino acids 14 - 52 of the FIV p10 protein upon translation.

9. The vaccine of claim 4 further comprising immunogens derived from viruses selected from the group consisting of feline leukemia virus, feline panleucopenia virus, feline rhinotracheitis virus, feline calicivirus, feline infectious peritoneal virus, feline herpesvirus, feline enteric coronavirus, or mixtures thereof.

10. The vaccine of claim 4 further comprising inactivated or attenuated feline *Chlamydia psittaci*, *Microsporium canis*, or mixtures thereof.

11. A FIV virion which does not comprise whole p10 nucleocapsid protein.

12. The FIV virion of claim 10 which was produced by transfection of appropriate host cells with a plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof.

13. Host cells which are transfected with a plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof, such that said cells produce FIV virions which do not comprise whole p10 nucleocapsid protein.

14. Transfected host cells of claim 12 which are selected from the group consisting of Vero cells (ATCC CCL 81), Crandell feline kidney cells (ATCC CCL 94), and AH927 feline embryonic fibroblast cells.

15. A method for preventing or lessening disease caused by Feline Immunodeficiency Virus (FIV), comprising administering to a feline in need of such treatment vaccine comprising FIV virions which do not comprise whole p10 nucleocapsid protein.

16. The method of claim 14 wherein said virion was produced by transfection of appropriate host cells with a plasmid encoding the FIV genome wherein the *gag* gene of said genome comprises a deletion of nucleotides encoding the nucleocapsid (p10) protein or a portion thereof.

17. The method of claim 14 wherein said deletion of nucleotides encoding the FIV p10 protein is as set out in Figure 2.

18. The method of claim 15 wherein said deletion encompasses nucleotides which results in the deletion of amino acids 14 - 52 of the FIV p10 protein upon translation.

1 / 4

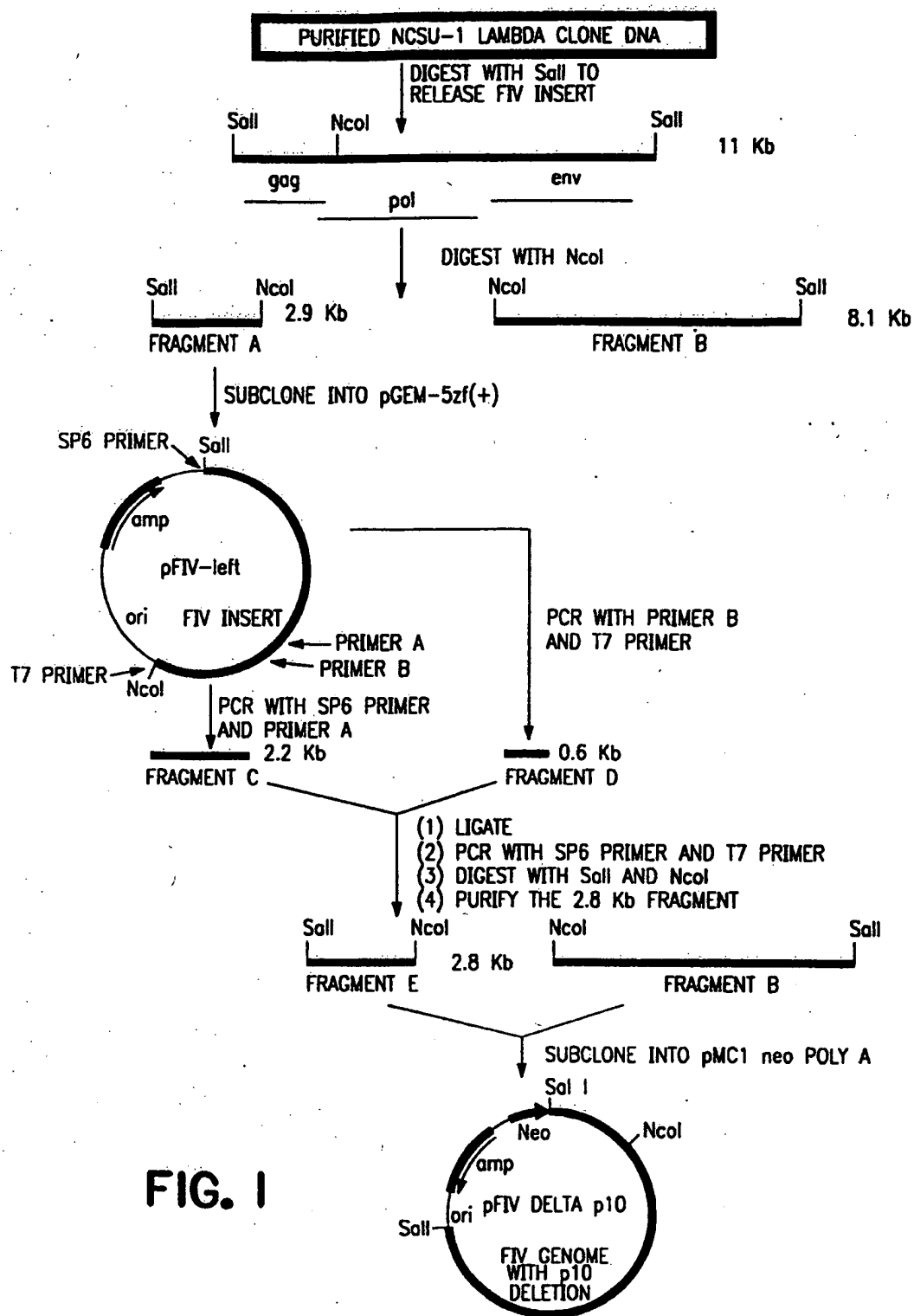


FIG. 1

SUBSTITUTE SHEET (RULE 26)

2 / 4

Sequence Range: 1 to 1353

10	20	30	40	50	60
ATGGGGAATG	GACAGGGGCG	AGATTGGAAA	ATGGCCATTA	AGAGATGTAG	TAATGCTGCT
TACCCCTTAC	CTGTCCCCGC	TCTAACCTTT	TACCGGTAAT	TCTCTACATC	ATTACGACGA
-----> p15 Matrix protein					
70	80	90	100	110	120
GTAGGAGTAG	GGGGGAAGAG	TAAAAAATTT	GGGGAAGGGA	ATTTTCAGATG	GGCCATTAGA
CATCCTCATC	CCCCCTTCTC	ATTTTTTAAA	CCCCTTCCCT	TAAAGTCTAC	CCGGTAATCT
130	140	150	160	170	180
ATGGCTAATG	TATCTACAGG	ACGAGAACCT	GGTGATATAC	CAGAGACTTT	AGATCAACTA
TACCGATTAC	ATAGATGTCC	TGCTCTTGGA	CCACTATATG	GTCTCTGAAA	TCTAGTTGAT
190	200	210	220	230	240
AGGTTGGTTA	TTTGCGATTT	ACAAGAAAGA	AGAAAAAAT	TTGGATCTTG	CAAAGAAATT
TCCAACCAAT	AAACGCTAAA	TGTTCTTTCT	TCTTTTTTTA	AACCTAGAAC	GTTTCTTTAA
250	260	270	280	290	300
GATAAGGCAA	TTGTTACATT	AAAAGTCTTT	GCGGCAGTAG	GACTTTTAAA	TATGACAGTG
CTATTCCGTT	AACAATGTAA	TTTTTCAGAAA	CGCCGTCATC	CTGAAAATTT	ATACTGTCAC
310	320	330	340	350	360
TCTTCTGCTG	CTGCAGCTGA	AAATATGTTT	ACTCAGATGG	GATTAGACAC	TAGACCATCT
AGAAGACGAC	GACGTCGACT	TTTATACAAG	TGAGTCTACC	CTAATCTGTG	ATCTGGTAGA
370	380	390	400	410	420
ATGAAAGAAG	CAGGAGGAAA	AGAGGAAGGC	CCTCCACAGG	CATTTCTTAT	TCAAACAGTA
TACTTTCTTC	GTCCTCCTTT	TCTCCTTCCG	GGAGGTGTCC	GTAAAGGATA	AGTTTGTGAT
p15 <-----> p25					
Capsid protein					
430	440	450	460	470	480
AATGGAGTAC	CACAAATATG	AGCACTTGAC	CCAAAAATGG	TGTCCATTTT	TATGGAAAAG
TTACCTCATG	GTGTTATACA	TCGTGAACGT	GGTTTTTACC	ACAGGTAAAA	ATACCTTTTC
490	500	510	520	530	540
GCAAGAGAAG	GATTAGGAGG	TGAGGAAGTT	CAGCTATGGT	TCACTGCCTT	CTCTGCAAAT
CGTTCTCTTC	CTAATCCTCC	ACTCCTTCAA	GTCGATACCA	AGTGACGGAA	GAGACGTTTA
550	560	570	580	590	600
TTAACACCTA	CTGACATGGC	CACATTAATA	ATGGCCGCAC	CAGGGTGCGC	TGCAGATAAA
AATTGTGGAT	GACTGTACCG	GTGTAATTAT	TACCGGCGTG	GTCCACGCG	ACGCTATTAT
610	620	630	640	650	660
GAAATATTGG	ATGAAAGCTT	AAAGCAACTT	ACTGCAGGAT	ATGATCGTAC	ACATCCCCCT
CTTTATAACC	TACTTTGAA	TTTCGTTGAA	TGACGTCCTA	TACTAGCATG	TGTAGGGGGA
670	680	690	700	710	720
GATGCTCCCA	GACCATTACC	CTATTTTACT	GCAGCAGAAA	TTATGGGTAT	TGGATTTACT
CTACGAGGGT	CTGGTAATGG	GATAAAATGA	CGTCGTCTTT	AATACCCATA	ACCTAAATGA

**FIG. 2A**  
SUBSTITUTE SHEET (RULE 26)



3/4

```

      730      740      750      760      770      780
      *      *      *      *      *      *
CAAGAACAAC AAGCAGAAGC AAGATTTGCA CCAGCTAGGA TGCAGTGTAG AGCATGGTAT
GTTCTTGTTG TTCGTCTTCG TTCTAAACGT GGTGATCCT ACGTCACATC TCGTACCATA

      790      800      810      820      830      840
      *      *      *      *      *      *
CTCGAGGGAC TAGGAAAATT GGGCGCCATA AAAGCTAAGT CTCCTCGAGC TGTGCAGTTA
GAGCTCCCTG ATCCTTTTAA CCCGCGGTAT TTTCGATTCA GAGGAGCTCG ACACGTCAAT

      850      860      870      880      890      900
      *      *      *      *      *      *
AGACAAGGAG CTAAGGAAGA TTATTCATCC TTTATTGACA GATTGTTTGC CCAAATAGAT
TCTGTTCCCTC GATTCCCTTCT AATAAGTAGG AAATAACTGT CTAACAAACG GGTATTATCTA

      910      920      930      940      950      960
      *      *      *      *      *      *
CAAGAACAAA ATACAGCTGA AGTTAAGTTA TATTTAAAAC AGTCATTAAAG CATGGCTAAT
GTTCTTGTTT TATGTCGACT TCAATTCAAT ATAAATTTTG TCAGTAATTC GTACCGATTA

      970      980      990      1000      1010      1020
      *      *      *      *      *      *
GCTAATGCAG AATGTAAAAA GCCAATGACC CACCTTAAGC CAGAAAGTAC CCTAGAAGAA
CGATTACGTC TTACATTTT CGGTTACTGG GTGGAATTCG GTCTTTCATG GGATCTTCTT

      1030      1040      1050      1060      1070      1080
      *      *      *      *      *      *
AAGTTGAGAG CTTGTCAAGA AATAGGCTCA CCAGGATATA AAATGCAACT CTTGGCAGAA
TTCAACTCTC GAACAGTTCT TTATCCGAGT GGTCCTATAT TTTACGTTGA GAACCGTCTT
p25 <-----

      1090      1100      1110      1120      1130      1140
      *      *      *      *      *      *
GCTCTTACAA AAGTTCAAGT AGTGCAATCA AAAGGATCAG GACCAGTGTG TTTTAATTGT
CGAGAATGTT TTCAAGTTCA TCACGTTAGT TTTCCTAGTC CTGGTCACAC AAAATTAACA
-----> p10 Nucleocapsid

      1150      1160      1170      1180      1190      1200
      *      *      *      *      *      *
AAAAAACCCAG GACATCTAGC AAGACAATGT AGAGAAGTGA GAAAATGTAA TAAATGTGGA
TTTTTTGGTC CTGTAGATCG TTCTGTTACA TCTCTTCACT CTTTACATT ATTACACCT

      1210      1220      1230      1240      1250      1260
      *      *      *      *      *      *
AAACCTGGTC ATGTAGCTGC CAAATGTTGG CAAGGAAATA GAAAGAATTC GGGAAACTGG
TTTGGACCAG TACATCGACG GTTTACAACC GTTCCTTTAT CTTTCTTAAG CCCTTTGACC

-----> POL

      1270      1280      1290      1300      1310      1320
      *      *      *      *      *      *
AAGGCGGGGC GAGCTGCAGC CCCAGTGAAT CAAGTGCAGC AAGCAGTAAT GCCATCTGCA
TTCCGCCCCG CTCGACGTCG GGGTCACTTA GTTCACGTCG TTCGTCATTA CGGTAGACGT

      1330      1340      1350
      *      *      *
CCTCCAATGG AGGAGAAACT ATTGGATTTA TAA
GGAGGTTACC TCCTCTTTGA TAACCTAAAT ATT
p10 <-----

```

POL -----&gt;

FIG. 2B

SUBSTITUTE SHEET (RULE 26)

4/4

Sequence Range: 1 to 1353

M G N G Q G R D W K M A I K R C S N A A  
 -----> p15 Matrix protein

V G V G G K S K K F G E G N F R W A I R  
 M A N V S T G R E P G D I P E T L D Q L  
 R L V I C D L Q E R R K K F G S C K E I  
 D K A I V T L K V F A A V G L L N M T V  
 S S A A A A E N M F T Q M G L D T R P S  
 M K E A G G K E E G P P Q A F P I Q T V

p15 <-----> p25  
 Capsid protein

N G V P Q Y V A L D P K M V S I F M E K  
 A R E G L G G E E V Q L W F T A F S A N  
 L T P T D M A T L I M A A P G C A A D K  
 E I L D E S L K Q L T A G Y D R T H P P  
 D A P R P L P Y F T A A E I M G I G F T  
 Q E Q Q A E A R F A P A R M Q C R A W Y  
 L E G L G K L G A I K A K S P R A V Q L  
 R Q G A K E D Y S S F I D R L F A Q I D  
 Q E Q N T A E V K L Y L K Q S L S M A N  
 A N A E C K K P M T H L K P E S T L E E  
 K L R A C Q E I G S P G Y K M Q L L A E

p25 &lt;-----&gt;

A L T K V Q V V Q S K G S G P V C F N C  
 -----> p10 Nucleocapsid

K K P G H L A R Q C R E V R K C N K C G

K P G H V A A K C W O G N R K N S G N W

K E F G K L  
 -----> POL (-1 ORF)

K A G R A A A P V N Q V Q Q A V M P S A  
 E G G A S C S P S E S S A A S S N A I C

P P M E E K L L D L  
 T S N G G E T I G F I

p10 <----->  
 POL ----->

FIG. 2C

SUBSTITUTE SHEET (RULE 26)

# INTERNATIONAL SEARCH REPORT

Int. Appl. No.  
PCT/US 96/08639

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC 6 C12N15/85 C12N15/49 C12N5/16 C12P21/00 A61K39/21		
According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC 6 C12N C12P A61K		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	VIROLOGY, vol. 183, 1991, pages 288-297, XP002013369 S. MORIKAWA ET AL.: "Analyses of the requirements for the synthesis of virus-like particles by FIV gag using baculovirus vectors" *see the whole article* ---	1-18
A	WO,A,93 01278 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA) 21 January 1993 ---	
A	US,A,5 275 813 (J.K. YAMAMOTO ET AL.) 4 January 1994 -----	
<input type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex.		
* Special categories of cited documents : <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&amp;" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search  <div style="text-align: center; font-size: 1.2em;">16 September 1996</div>		Date of mailing of the international search report  <div style="text-align: center; font-size: 1.2em;">30.10.96</div>
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016		Authorized officer  <div style="text-align: center; font-size: 1.2em;">Marie, A</div>

**INTERNATIONAL SEARCH REPORT**

Information on patent family members

International Application No

PCT/US 96/08639

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9301278	21-01-93	AU-A- 2299892	11-02-93
		US-A- 5510106	23-04-96
-----			
US-A-5275813	04-01-94	US-A- 5037753	06-08-91
		US-A- 5510106	23-04-96
		US-A- 5118602	02-06-92
-----			